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INTERACTION BETWEEN VEE VIRUS AND RICKETTSIA RICKETTSII IN SUSPENDED L CELLS

Henry R. Tribble, Jr.

Horace B. Rees, Jr.

Henry J. Hearn, Jr.

Virus and Rickettsia Division
and
Medical Bacteriology Division
DIRECTOR OF BIOLOGICAL RESEARCH

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ABSTRACT

Rickettsia rickettsii were found to interfere with the growth of Venezuelan equine encephalitis virus in suspended L cell cultures grown in a defined medium. The effectiveness of the inhibition of viral growth was most pronounced in cultures in which the rickettsiae were introduced 2 days prior to the virus. Under these conditions, virus exhibited only minimal signs of growth. In cultures infected simultaneously the virus achieved its maximal titer but underwent an unusually rapid cessation of growth soon afterwards. The viral titer of L cell cultures chronically infected with VEE virus for 86 days disappeared 4 to 5 days after the inoculation of rickettsiae. Possible differences in susceptibility to virus and rickettsiae among the cells in culture were implicated as playing a role in this phenomenon. In support of this, virus multiplied in cat kidney cells to higher titers and rickettsiae to lower titers than in L cells. In the cat kidney cells, the rickettsiae failed to inhibit the growth of virus. The failure of virus to grow in 2-day-old rickettsial-infected L cell cultures, however, suggests that under the proper conditions the latter organism was capable of influencing the entire culture against viral growth. No viral inhibitor, however, was demonstrated in cell-free preparations as a result of the rickettsial infection.

INTERACTION BETWEEN VEE VIRUS AND *RICKETTSIA RICKETTSII* IN SUSPENDED L CELLS

The proliferation of Venezuelan equine encephalomyelitis (VEE) virus and *Rickettsia rickettsii* in L cell suspension cultures grown in defined medium has been reported previously. This paper presents data on the interaction of VEE virus and *R. rickettsii* in the same cell culture. The composition of the defined medium used in these experiments has been presented previously. Very briefly, it contains salts, glucose, 13 amino acids, vitamins, and additives such as methocel antibiotics and phenol red. One hundred units of penicillin and 20 micrograms of streptomycin sulfate per ml were also included in this medium after *R. rickettsii* was shown to grow in the presence of these antibiotic concentrations.

The experiments were conducted in 30-ml amounts in 100-ml serum bottles in a New Brunswick Gyrotory shaker at 35 C. L cells were obtained from stock cultures and grown in suspension in the defined medium mentioned above. The culture medium was renewed at 48 hour intervals. The virus inoculum was our stock egg seed strain that was derived from an infected donkey. *R. rickettsii* was a partially purified yolk sac preparation of the Bitteroot strain. The virus and/or rickettsia was inoculated into suspensions of approximately 5×10^5 L cells per ml and allowed to incubate for 45 minutes. The cells were then washed. Samples obtained at various intervals postinoculation for the assay of either organism were stored at -60 C. Assays of the *R. rickettsii* preparations were performed in 7-day-old embryonated chicken eggs and the results expressed as yolk sac LD_{50} ($YSLD_{50}$) per ml. Assays of the VEE virus preparations were performed in 10- to 14-gram mice* by the intracerebral (ic) route and the results expressed as $MICLD_{50}$ per ml.

Prior to determinations on the growth of VEE virus or *R. rickettsii* in L cell cultures, tests were performed to ascertain the inactivation of each microorganism in medium alone. These results are shown in Figure 1. From a starting titer of 10^8 $MICLD_{50}$/ml, VEE virus infectivity declined to an undetectable level within 3 days. *R. rickettsii* was extremely unstable, and a rickettsial suspension lost 4 or more logs of egg infectivity in 12 hours at 35 C. In order to assess the effect of an interaction between the virus and rickettsiae in culture, a brief review of certain kinetic aspects of their respective growth cycles independent of one another is necessary.

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* In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

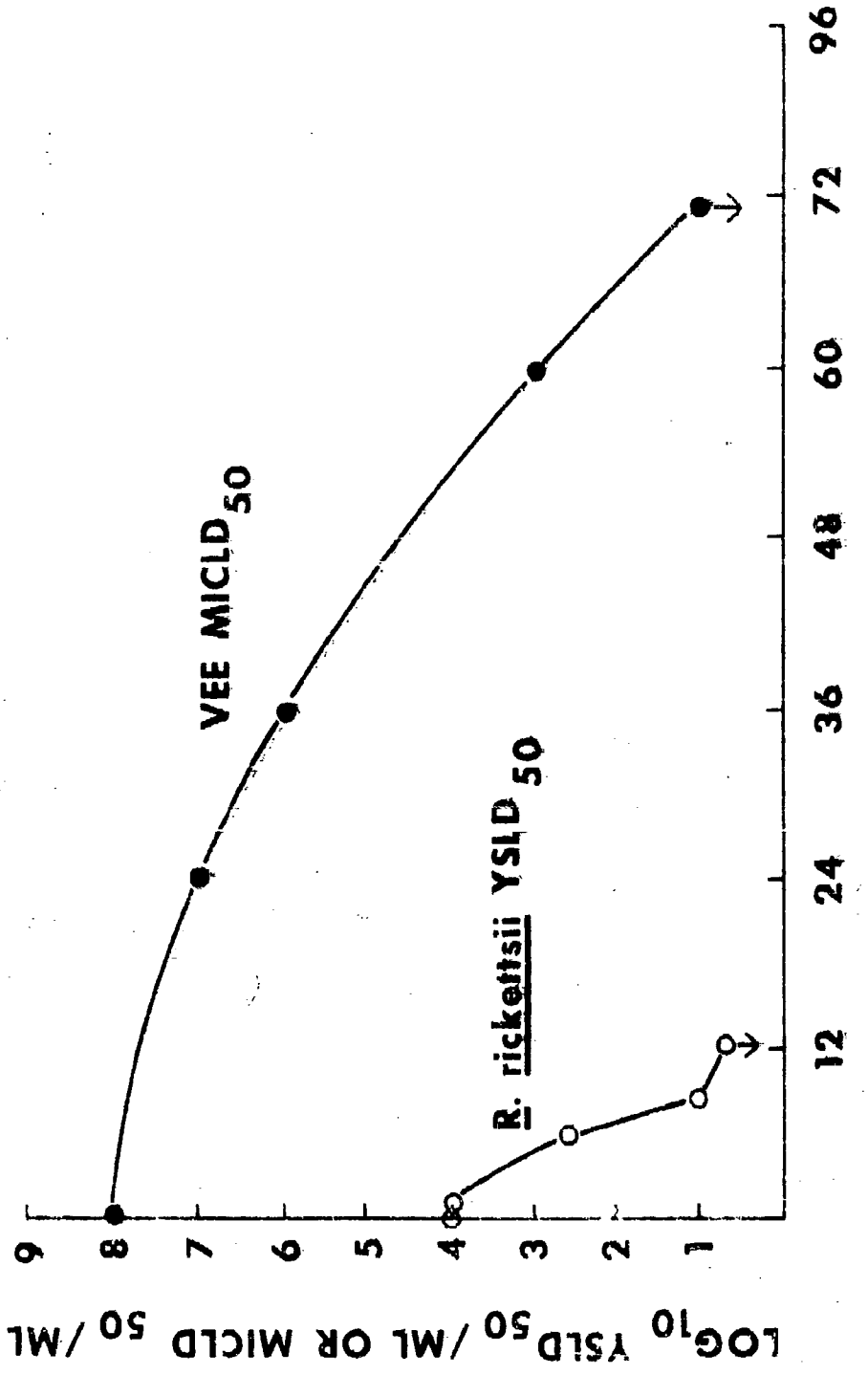

Figure 1. Inactivation of VEE Virus and R. rickettsii in Culture Media.

VEE virus and R. rickettsii growth curves that are typical of several experiments with L cells suspended in defined medium are presented in Figure 2. VEE virus demonstrated a maximal titer of $10^{7.3}$ $MICLD_{50}$ at day 1. At day 2, the titer remained near its maximal level. By day 5, the virus titers uniformly declined to approximately 10^3 $MICLD_{50}$. R. rickettsii proliferated to its maximal titer of $10^{5.1}$ $YSLD_{50}$/ml at day 2. At subsequent 24-hour intervals the titers gradually declined until values of approximately 10^3 $YSLD_{50}$ were obtained on day 7. Cell lysis that did not involve the entire culture was commonly encountered during studies with both organisms.

The interaction of virus and rickettsiae was studied by examining the growth response of both microorganisms within the same culture. Three experimental test plans were used: one in which the virus and the rickettsiae were inoculated simultaneously, a second in which the virus was inoculated two days after the rickettsiae, and a third in which the rickettsiae were inoculated into a culture of L cells that were chronically infected with VEE virus.

Results obtained after the simultaneous inoculation of VEE virus and R. rickettsii into an L cell culture are shown in Figure 3. VEE virus achieved a maximal level of growth of 10^7 $MICLD_{50}$/ml at day 1. By day 2, however, slight alterations began to appear, suggesting that the rickettsiae were beginning to influence the growth activity of the virus. By day 3 a change from the typical pattern of growth of VEE virus was clearly demonstrated. Titers of approximately 10^3 $MICLD_{50}$ were present, representing levels that were at least 100-fold lower than those shown by cultures inoculated with the virus alone. Moreover, by day 3, rickettsial titers exceeded the viral titers, which remained at comparatively low levels until day 5 when no virus could be detected. Note that rickettsial titers (Figure 3) such as those found at day 3 and beyond could be obtained only when the titers of the virus in the culture were sufficiently low for the rickettsiae to express their lethal effect when assayed in eggs. In such cases, embryonated eggs were killed within 48 hours upon injection with low dilutions of the samples that contained virus. With higher dilutions of the same sample, in which the virus was then diluted out, the rickettsiae were lethal for eggs at 7 to 10 days. Thus, in many cases, the rickettsial lethality that occurred in embryonated eggs during an assay of a mixture of virus and rickettsiae was easily distinguished. A comparison between the rickettsial titers in Figure 3 and those obtained when the rickettsiae were grown without virus indicates that the growth activity of the rickettsiae was not altered in the presence of virus.

Under the second set of experimental conditions, R. rickettsii was inoculated into the culture 2 days prior to the virus. In this case, the results of which are shown in Figure 4, the virus multiplied only very slightly. In contrast to situations in which the virus only was

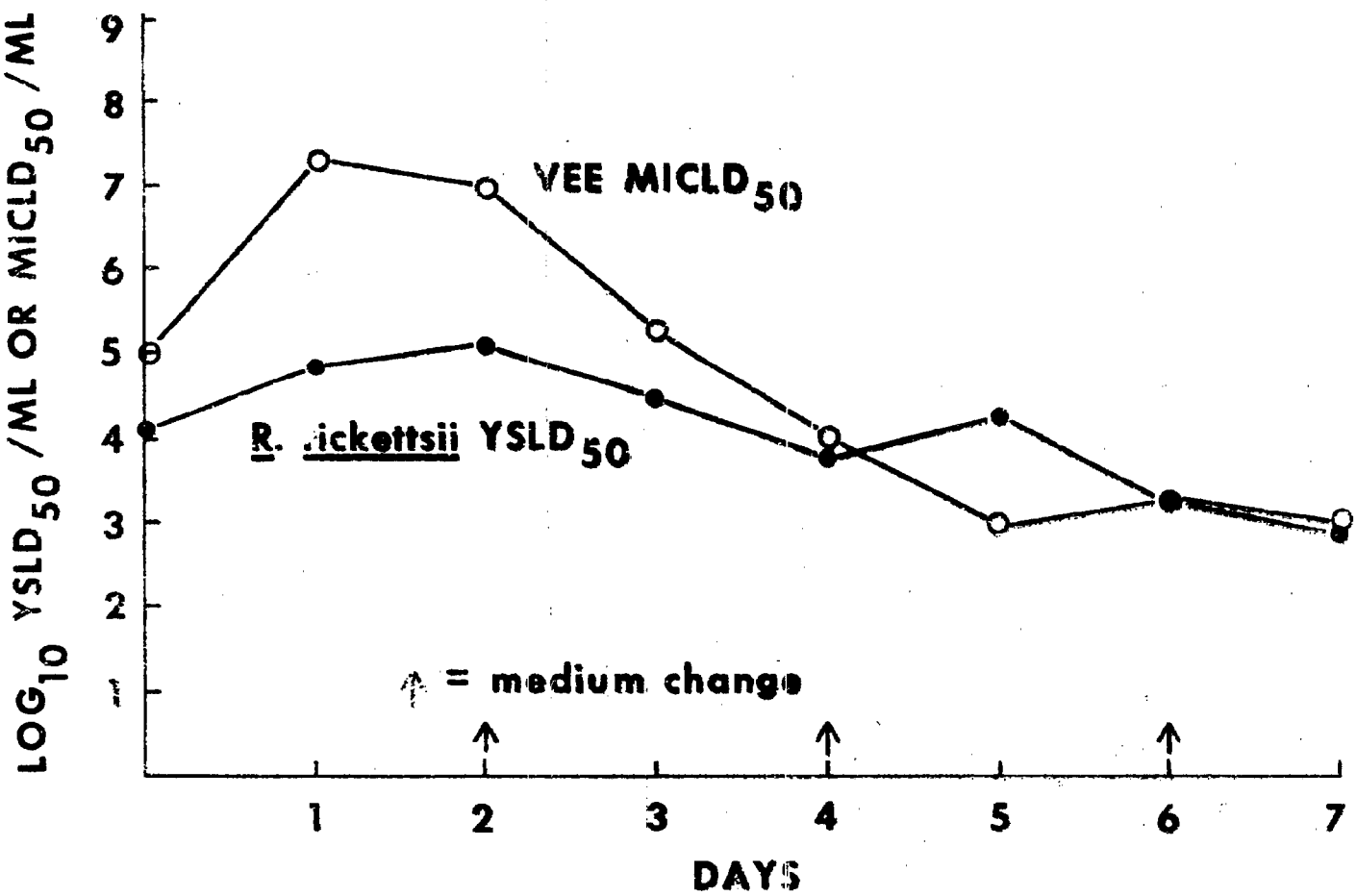

Figure 2. Growth of R. rickettsii and VEE Virus in Suspended L Cell Cultures.

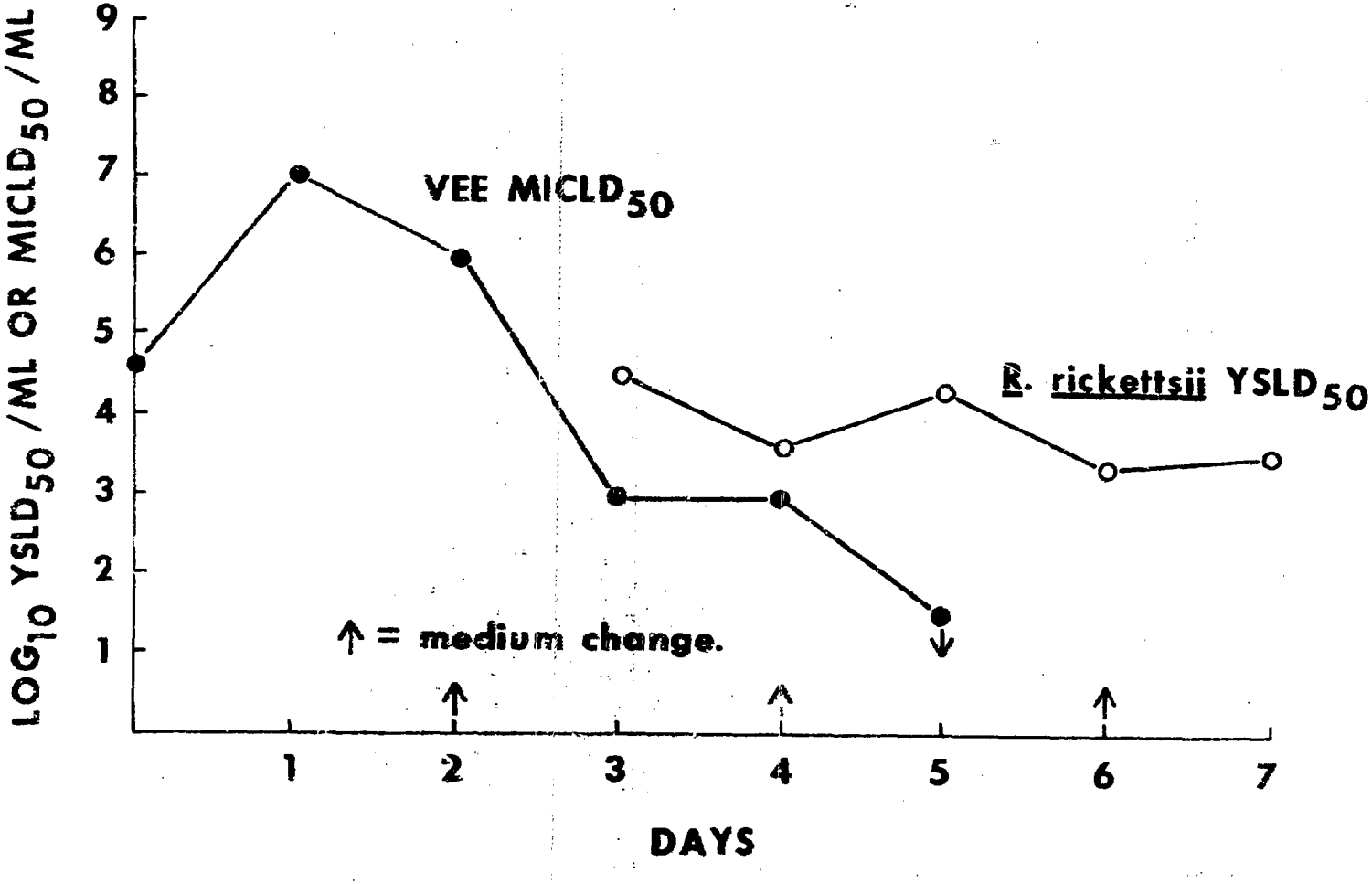

Figure 3. Growth of R. rickettsii and VEE Virus Inoculated Simultaneously in Suspended L Cell Culture.

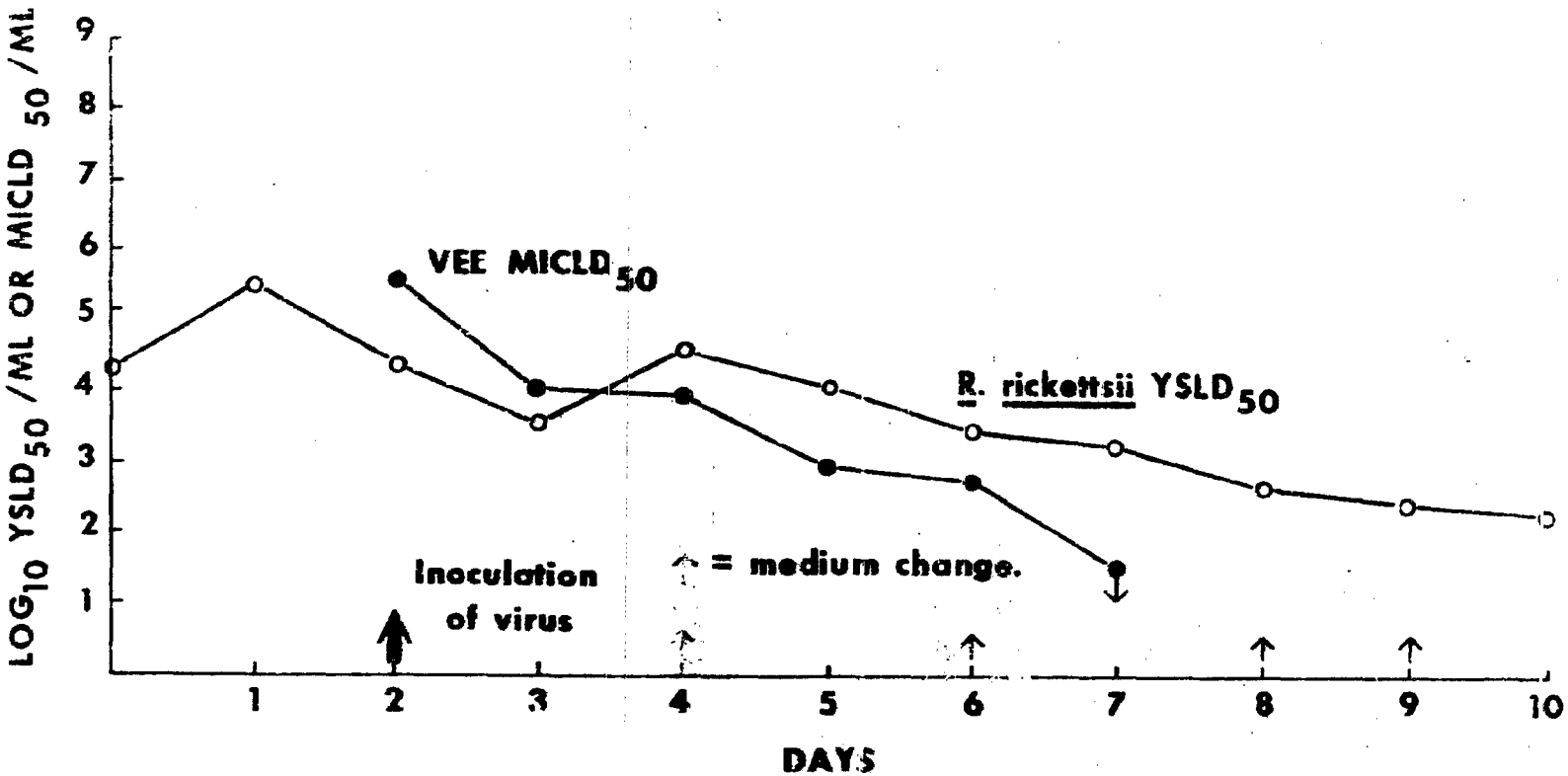

Figure 4. Growth of VEE Virus Inoculated Two Days after R. rickettsii in Suspended L Cell Culture.

present in culture or when the virus and rickettsiae were inoculated together, the initial titer of approximately 10^5 $MICLD_{50}$ at day 0 was not exceeded at any subsequent time period. Instead, the recoveries of virus steadily declined at a rate that was, in fact, only slightly slower than that found for the virus in medium alone at 35 C. Clearly, R. rickettsii prevented the growth of the virus with little if any alteration in its own pattern of growth.

In view of the previous results that demonstrated that R. rickettsii is an efficient inhibitor of viral growth, the third set of experimental conditions was tested. L cells in which the virus was well established as a chronic infection (for 86 days) were tested for their repsonse to a superinfection with the rickettsiae. The results of this test are shown in Figure 5.

Within the first 3 days postinoculation of the rickettsiae, the quantities of virus that were routinely present appeared to be sufficient to occlude the rickettsiae. This suggested that the rickettsiae had some difficulty in establishing its infection in the cells chronically infected with VEE virus. Within 5 days, however, the virus was no longer detectable in the culture. The rickettsiae apparently suppressed viral synthesis and subsequently displayed a pattern of growth that was similar to those cultures infected with rickettsiae alone.

Investigations on the possible mechanisms by which R. rickettsii may inhibit viral growth are continuing. Briefly, the question of whether different cell types may more efficiently support one or the other organism was considered. For example, the results could possibly be construed to indicate that the L cell population may be heterogeneous, with one portion of the cells favorable for viral growth and another favorable for rickettsial growth. Cells favorable to the rickettsial propagation survived and became the sole inhabitants of the culture because the virus rapidly destroyed the cells that it had invaded. Evidence that tends to support this line of reasoning can be found in a study with a different line of cells, namely, cat kidney cells that supported viral growth at higher titers and rickettsial growth at lower titers than the L cells. In contrast, interference with viral growth by the rickettsiae was not observed in the cat kidney cells. The theory of cell selection does not explain, however, why the virus growth was limited when inoculated into the L cells 2 days after the rickettsiae unless it is further postulated that during rickettsial growth, cells that were ordinarily susceptible to virus were prevented from performing in that capacity. An exogenously produced inhibitor or interferon-like substance could account for the rickettsial antagonism against virus, but no such substance has yet been demonstrated in cell-free preparations. Other mechanisms, such as an unsuccessful competition by the virus for substance utilized during rickettsial growth or an interference in which the rickettsiae exclude the virus from penetrating the cell, are currently being considered.

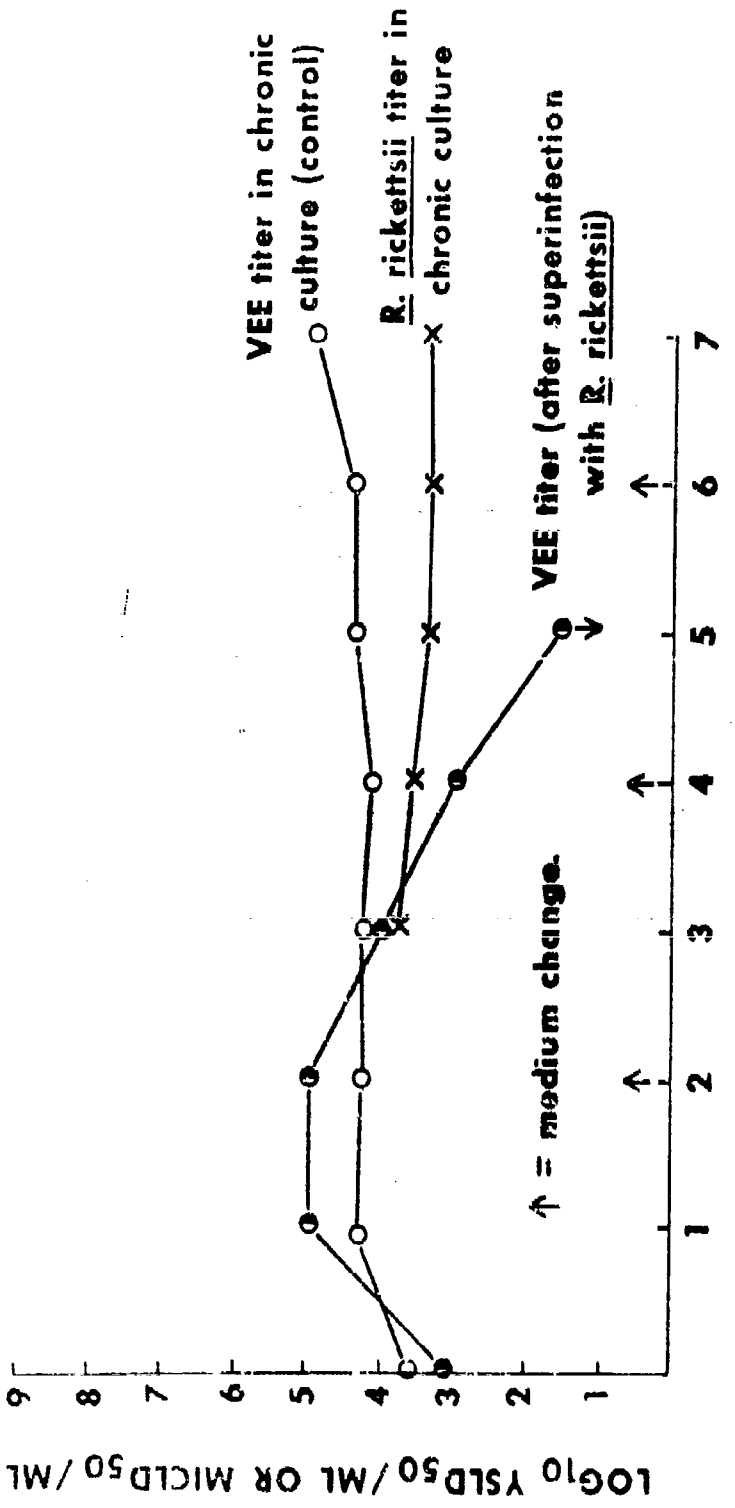

Figure 5. Effect of R. rickettsii upon Suspended L Cell Culture Chronically Infected with VEE Virus.

In summary, Rickettsia rickettsii interfered with the growth of Venezuelan equine encephalomyelitis (VEE) virus in suspended L cell cultures grown in a defined medium. The effectiveness of the inhibition of viral growth was most pronounced in cultures in which the rickettsiae were introduced 2 days prior to the virus. Under these conditions, virus exhibited only minimal signs of growth. In simultaneously infected cultures, the virus achieved its maximal titer but underwent an unusually rapid cessation of growth soon afterwards. The viral titer of L cell cultures chronically infected with VEE virus for 86 days disappeared 4 to 5 days after the inoculation of the rickettsiae. Possible differences in susceptibility to virus and rickettsiae among the cells in culture were implicated as playing a role in this phenomenon. In support of this, virus multiplied in cat kidney cells to higher titers and rickettsiae to lower titers than in L cells. In the cat kidney cells, the rickettsiae failed to inhibit the growth of virus. The failure of virus to grow in 2-day-old rickettsial-infected L cell cultures, however, suggests that under the proper conditions the latter organism was capable of influencing the entire culture against viral growth. No viral inhibitor, however, was demonstrated in cell-free preparations as a result of the rickettsial infection.